10/634,314

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     (FILE 'HOME' ENTERED AT 15:20:11 ON 12 SEP 2005)

     FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH, LIFESCI' ENTERED AT 15:22:09 ON
     12 SEP 2005
L1         136934 S LIBRARY(5A)MUTATION OR (INSERT? OR SITE-DIRECT?)(6A)MUTAGENES
L2          59644 S TRANSPOSON OR TRANSPOSASE OR MINOS OR MARINER OR HERMES OR PI
L3           1775 S L1(S)L2
L4           1556 S L1(9A)L2
L5        2104632 S VIRUS OR (VIRAL OR RETROVIRAL OR LENTIVIRAL OR ADENOVIRAL OR
L6             99 S L3 AND L5
L7             27 S L3(S)L5
L8             62 DUP REM L6 (37 DUPLICATES REMOVED)
L9             21 DUP REM L7 (6 DUPLICATES REMOVED)

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L9 ANSWER 1 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN

AU Moradpour, Darius; Evans, Matthew J.; Gosert, Rainer; Yuan, Zhenghong; Blum, Hubert E.; Goff, Stephen P.; Lindenbach, Brett D.; Rice, Charles M.

TI Insertion of green fluorescent protein into nonstructural protein 5A allows direct visualization of functional hepatitis C virus replication complexes

SO Journal of Virology (2004), 78(14), 7400-7409
CODEN: JOVIAM; ISSN: 0022-538X

AB Hepatitis C virus (HCV) replicates its genome in a membrane-associated replication complex, composed of viral proteins, replicating RNA and altered cellular membranes. The authors describe here HCV replicons that allow the direct visualization of functional HCV replication complexes. Viable replicons selected from a library of Tn7-mediated random insertions in the coding sequence of nonstructural protein 5A (NS5A) allowed the identification of two sites near the NS5A C terminus that tolerated insertion of heterologous sequences. Replicons encoding green fluorescent protein (GFP) at these locations were only moderately impaired for HCV RNA replication. Expression of the NS5A-GFP fusion protein could be demonstrated by immunoblot, indicating that the GFP was retained during RNA replication and did not interfere with HCV polyprotein processing. More importantly, expression levels were robust enough to allow direct visualization of the fusion protein by fluorescence microscopy. NS5A-GFP appeared as brightly fluorescing dot-like structures in the cytoplasm. By confocal laser scanning microscopy, NS5A-GFP colocalized with other HCV nonstructural proteins and nascent viral RNA, indicating that the dot-like structures, identified as membranous webs by electron microscopy, represent functional HCV replication complexes. These findings reveal an unexpected flexibility of the C-terminal domain of NS5A and provide tools for studying the formation and turnover of HCV replication complexes in living cells.

L9 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN

IN Harrington, John Joseph; Jackson, Paul David; Jiang, Li

TI Methods for mutating genes in cells and animals using retroviral vector insertional mutagenesis

SO PCT Int. Appl., 132 pp.
CODEN: PIXXD2

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 2003040324 | A2 | 20030515 | WO 2002-US35405 | 20021104 |
| | WO 2003040324 | A3 | 20031211 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,

PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---|---|---|---|---|
| US 2003134421 | A1 | 20030717 | US 2002-288555 | 20021104 |
| EP 1451295 | A2 | 20040901 | EP 2002-780573 | 20021104 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

AB The present invention is in the fields of mol. biol., cell biol., and genetics. The invention is directed generally to mutating genes in cells in vitro and in multi-cellular organisms. The invention encompasses methods for mutating genes in cells using polynucleotides that act as insertional mutagens. Such methods are used to achieve mutation of a single gene to achieve a desired phenotype as well as mutation of multiple genes, required cumulatively to achieve a desired phenotype, in a cell or in a multi-cellular organism. The invention is also directed to methods of identifying one or more mutated genes, made by the methods of the invention, in cells and in multi-cellular organisms, by means of a tagging property provided by the insertional mutagen(s). The insertional mutagen thus allows identification of one or more genes that are mutated by insertion of an insertional mutagen. The invention is also directed to methods for correlating a phenotype with a gene by screening or selecting cells that have been mutated by an insertional mutagen incorporated into one or more genes in a cell and identifying the gene or genes causing the phenotype by means of a tagging property in one or more of the insertional mutagens. The invention is also directed to cells and multi-cellular organisms created by the methods of the invention and uses of the cells and multicellular organisms. The invention is also directed to libraries of cells created by the methods of the invention and uses of the libraries. An exemplary vector pDKO2 designed to trap transcriptionally active genes using the function of the splice acceptor in the vector is described. PDKO2 (3'LTR-lox-S/A-x-IRES-DR-bGHpA-TK-PGK-lox-Y-5'LTR) contains vector backbone from self-inactivating retroviral vector pSIR and genetic elements including S/A:branch site and splice acceptor (from the intron of an immunoglobin gene heavy chain variable region), lox:lox71/lox66 sequences, cre recombinase recognition sites, x:stop codons in all 3 reading frames, IRES: wild type internal ribosomal entry site from EMCV, DR:drug resistance gene for selection in the presence of neomycin, bGHpA: bovine growth hormone polyA sequence, TK: thymidine kinase, PGK: PGK promoter, and Φ:retrovirus packaging signal. Theor., when the vector is integrated into a gene, splicing can occur using endogenous splice donor at the end of exons and the splice acceptor provided by the vector. Once this splicing event occurs, a fusion transcript will be made resulting in a truncated protein of the trapped gene. IRES enables the expression of the drug selection marker when an active promoter is trapped, which allows selection of gene trap event. The retrovirus produced from RetroPack PT67 cells is used to infect Jurkat and gene trapping is detected by RT-PCR in genes for DHFR, HPRT, FasR, and Casp8 using their specific primers.

L9 ANSWER 3 OF 21 CAPLUS  DUPLICATE 1
AU Vilen, Heikki; Aalto, Juha-Matti; Kassinen, Anna; Paulin, Lars; Savilahti, Harri
TI A direct transposon insertion tool for modification and functional analysis of viral genomes
SO Journal of Virology (2003), 77(1), 123-134
CODEN: JOVIAM; ISSN: 0022-538X
AB Advances in DNA transposition technol. have recently generated efficient tools for various types of functional genetic analyses. The authors demonstrate here the power of the bacteriophage Mu-derived in vitro DNA transposition system for modification and functional characterization of a

complete bacterial virus genome. The linear double-stranded DNA genome of Escherichia coli bacteriophage PRD1 was studied by insertion mutagenesis with reporter mini-Mu transposons that were integrated in vitro into isolated genomic DNA. After introduction into bacterial cells by electroporation, recombinant transposon-containing virus clones were identified by autoradiog. or visual blue-white screening employing α-complementation of E. coli β-galactosidase. Addnl., a modified transposon with engineered NotI sites at both ends was used to introduce novel restriction sites into the phage genome. Anal. of the transposon integration sites in the genomes of viable recombinant phage generated a functional map, collectively indicating genes and genomic regions essential and nonessential for virus propagation. Moreover, promoterless transposons defined the direction of transcription within several insert-tolerant genomic regions. These strategies for the anal. of viral genomes are of a general nature and therefore may be applied to functional genomics studies in all prokaryotic and eukaryotic cell viruses.

L9 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
IN Savakis, Charalambos; Grosveld, Frank
TI Transposon-based insertional mutagenesis for producing a mutational library
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 2002062991 | A1 | 20020815 | WO 2002-GB484 | 20020205 |
| | W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| | RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| | CA 2437309 | AA | 20020815 | CA 2002-2437309 | 20020205 |
| | EP 1358321 | A1 | 20031105 | EP 2002-710174 | 20020205 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| | US 2004092018 | A1 | 20040513 | US 2003-634314 | 20030805 |

AB The invention provides a method for producing a **library** of genetic **mutations** in a cell population by **insertional mutagenesis**, wherein a **viral vector** comprising a **transposon** is used to deliver said **transposon** to said cell population, which cell population stably expresses the cognate **transposase** for said **transposon**, and the **transposon** is mobilized to give rise to the genetic mutations. Examples 1 and 2 describe the use of baculovirus for high efficiency introduction of transposons into cells expressing transposase using pMiLRgeo and pMiLRneo resp. Examples 3 and 4 describes the use of retrovirus vectors for introduction of transposons into cells expressing transposase.

L9 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
AU Kumar, Anuj; Vidan, Susana; Snyder, Michael
TI Insertional mutagenesis: transposon-insertion libraries as mutagens in yeast
SO Methods in Enzymology (2002), 350(Guide to Yeast Genetics and Molecular and Cell Biology, Part B), 219-229
CODEN: MENZAU; ISSN: 0076-6879
AB Comprehensive protocols for using insertional libraries as mutagens in

yeast are provided. A plasmid-based library of transposon-mutagenized yeast DNA is used to generate and identify the target yeast mutants. By employing insertional libraries carrying a specially designed multipurpose transposon, insertions are modified in yeast to generate corresponding epitope-tagged alleles for various functional studies. (c) 2002 Academic Press.

L9 ANSWER 6 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Nakai, H.; Yant, S.R.; Storm, T.A.; Fuess, S.; Meuse, L.; Kay, M.A.*
V TI Extrachromosomal Recombinant Adeno-Associated Virus Vector Genomes Are Primarily Responsible for Stable Liver Transduction In Vivo
SO Journal of Virology [J. Virol.], (20010800) vol. 75, no. 15, pp. 6969-6976.
ISSN: 0022-538X.
AB Recombinant adeno-associated **virus** (rAAV) vectors stably transduce hepatocytes in experimental animals. Although the vector genomes are found both as extrachromosomes and as chromosomally integrated forms in hepatocytes, the relative proportion of each has not yet been clearly established. Using an in vivo assay based on the induction of hepatocellular regeneration via a surgical two-thirds partial hepatectomy, we have determined the proportion of integrated and extrachromosomal rAAV genomes in mouse livers and their relative contribution to stable gene expression in vivo. Plasma human coagulation factor IX (hF.IX) levels in mice originating from a chromosomally integrated hF.IX-expressing **transposon** vector remained unchanged with hepatectomy. This was in sharp contrast to what was observed when a surgical partial hepatectomy was performed in mice 6 weeks to 12 months after portal vein injection of a series of hF.IX-expressing rAAV vectors. At doses of 2.4 x 10 super(11) to 3.0 x 10 super(11) vector genomes per mouse (n = 12), hF.IX levels and the average number of stably transduced vector genomes per cell decreased by 92 and 86%, respectively, after hepatectomy. In a separate study, one of three mice injected with a higher dose of rAAV had a higher proportion (67%) of integrated genomes, the significance of which is not known. Nevertheless, in general, these results indicate that, in most cases, no more than 10% of stably transduced genomes integrated into host chromosomes in vivo. Additionally, the results demonstrate that extrachromosomal, not integrated, genomes are the major form of rAAV in the liver and are the primary source of rAAV-mediated gene expression. This small fraction of integrated genomes greatly decreases the potential risk of vector-related **insertional mutagenesis** associated with all integrating vectors but also raises uncertainties as to whether rAAV-mediated hepatic gene expression can persist lifelong after a single vector administration.

V L9 ANSWER 7 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Hobom, U.; Brune, W.; Messerle, M.; Hahn, G.; Koszinowski, U.H.*
TI Fast Screening Procedures for Random Transposon Libraries of Cloned Herpesvirus Genomes: Mutational Analysis of Human Cytomegalovirus Envelope Glycoprotein Genes
SO Journal of Virology [J. Virol.], (20000900) vol. 74, no. 17, pp. 7720-7729.
ISSN: 0022-538X.
AB We have cloned the human cytomegalovirus (HCMV) genome as an infectious bacterial artificial chromosome (BAC) in Escherichia coli. Here, we have subjected the HCMV BAC to random **transposon** (Tn) **mutagenesis** using a Tn1721-derived **insertion** sequence and have provided the conditions for excision of the BAC cassette. We report on a fast and efficient screening procedure for a Tn insertion library. Bacterial clones containing randomly mutated full-length HCMV genomes were transferred into 96-well microtiter plates. A PCR screening method based on two Tn primers and one primer specific for the desired genomic position of the Tn insertion was established. Within three consecutive rounds of PCR a Tn insertion of interest can be assigned to a

specific bacterial clone. We applied this method to retrieve mutants of HCMV envelope glycoprotein genes. To determine the infectivities of the mutant HCMV genomes, the DNA of the identified BACs was transfected into permissive fibroblasts. In contrast to BACs with mutations in the genes coding for gB, gH, gL, and gM, which did not yield infectious **virus**, BACs with disruptions of open reading frame UL4 (gp48) or UL74 (gO) were viable, although gO-deficient **viruses** showed a severe growth deficit. Thus, gO (UL74), a component of the glycoprotein complex III, is dispensable for viral growth. We conclude that our approach of PCR screening for Tn insertions will greatly facilitate the functional analysis of herpesvirus genomes.

L9 ANSWER 8 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Matzke, M.; Mette, M.; Matzke, A.
TI Transgene silencing by the host genome defense: implications for the evolution of epigenetic control mechanisms in plants and vertebrates
SO Plant Molecular Biology [Plant Mol. Biol.], (20000600) vol. 43, no. 2-3, pp. 401-415.
ISSN: 0167-4412.
AB Increasing evidence supports the idea that various transgene silencing phenomena reflect the activity of diverse host defense responses that act ordinarily on natural foreign or parasitic sequences such as transposable elements, viroids, RNA and DNA **viruses**, and bacterial DNA. Transgenes or their transcripts can resemble these cellular invaders in a number of ways, thus making them targets of host protective reactions. At least two distinct host defense systems operate to silence transgenes. One acts at the genome level and is associated with de novo DNA methylation. A second line of defense operates post-transcriptionally and involves sequence-specific RNA degradation in the cytoplasm. Transgenes that are silenced as a consequence of the genome defense are revealing that de novo methylation can be cued by DNA-DNA or RNA-DNA interactions. These methylation signals can be interpreted in the context of transposable elements or their transcripts. During evolution, as transposable elements accumulated in plant and vertebrate genomes and as they invaded flanking regions of genes, the genome defense was possibly recruited to establish global epigenetic mechanisms to regulate gene expression. **Transposons** integrated into promoters of host genes could conceivably change expression patterns and attract methylation, thus imposing on endogenous genes the type of epigenetic regulation associated with the genome defense. This recruitment process might have been particularly effective in the polyploid genomes of plants and early vertebrates. Duplication of the entire genome in polyploids buffers against **insertional mutagenesis** by transposable elements and permits their infiltration into individual copies of duplicated genes.

L9 ANSWER 9 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
AU Chin, Hang Gyeong; Park, Sung Han; Choe, Mi Sook; Park, Su Hyun; Oh, Byeong Keun; Lee, Gi Hwan; Choe, Hae Choon; Cho, Moo Je; Hong, Jong Chan; Han, Chang-deok
TI Germinal virus vector WDV (wheat dwarf virus)-mediated multiple insertions of a maize transposon, Ds (dissociation), in rice
SO Journal of Plant Biology (2000), 43(1), 1-9
CODEN: JPBIEZ; ISSN: 1226-9239
AB Wheat dwarf virus (WDV) is a monocot-infecting geminivirus that replicates in infected tissue as double-stranded DNA. We evaluated whether the WDV vector system bearing Ds could be used as an effective insertional mutagen in rice. Mol. data showed that Ds was excised from WDV vectors once the WDV-carrying Ds (WDV::Ds) and the genomic Ac vector were co-introduced into rice calli. Mature T0 and T1 transgenic plants were analyzed for the distribution and inheritance of Ds inserts. Southern anal. indicated that the Ds elements excised from WDV vectors were stably inserted into genomes. The number of transposed Ds ranged from zero to three copies, among

independent transformants. Meanwhile, untransposed Ds (WDV::Ds) were present in multiple-copies in genomes. Southern anal. of the selfed progeny of T0 plants demonstrated that most WDV::Ds were co-segregated among siblings. This indicated that these elements were integrated into the same single loci. However, a few Ds were found to segregate independently from the majority of Ds. In this report, we discuss the efficiency of WDV vectors in generating multicopy Ds in rice genomes.

√ L9 ANSWER 10 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Brune, W.; Menard, C.; Hobom, U.; Odenbreit, S.; Messerle, M.; Koszinowski, U.H.*
TI Rapid identification of essential and nonessential herpesvirus genes by direct transposon mutagenesis
SO Nature Biotechnology [Nat. Biotechnol.], (19990400) vol. 17, no. 4, pp. 360-364.
ISSN: 1087-0156.
AB Herpesviruses are important pathogens in animals and humans. The large DNA genomes of several herpesviruses have been sequenced, but the function of the majority of putative genes is elusive. Determining which genes are essential for their replication is important for identifying potential chemotherapy targets, designing herpesvirus vectors, and generating attenuated vaccines. For this purpose, we recently reported that herpesvirus genomes can be maintained as infectious bacterial artificial chromosomes (BAC) in Escherichia coli. Here we describe a one-step procedure for random-**insertion mutagenesis** of a herpesvirus BAC using a Tn1721-based **transposon** system. **Transposon** insertion sites were determined by direct sequencing, and infectious **virus** was recovered by transfecting cultured cells with the mutant genomes. Lethal mutations were rescued by cotransfecting cells containing noninfectious genomes with the corresponding wild-type subgenomic fragments. We also constructed revertant genomes by allelic exchange in bacteria. These methods, which are generally applicable to any cloned herpesvirus genome, will facilitate analysis of gene function for this **virus** family.

L9 ANSWER 11 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU DeAngelis, P.L.; Jing, Wei; Drake, R.R.; Achyuthan, A.M.
TI Identification and molecular cloning of a unique hyaluronan synthase from Pasteurella multocida
SO J. BIOL. CHEM., (19980400) vol. 273, no. 14, pp. 8454-8458.
ISSN: 0021-9258.
AB Type A Pasteurella multocida, a prevalent animal pathogen, employs a hyaluronan [HA] polysaccharide capsule to avoid host defenses. We utilized **transposon insertional mutagenesis** to identify the P. multocida HA synthase, the enzyme that polymerizes HA. A DNA fragment from a wild-type genomic library could direct HA production in vivo in Escherichia coli, a bacterium that normally does not produce HA. Analysis of truncated plasmids derived from the original clone indicated that an open reading frame encoding a 972-residue protein was responsible for HA polymerization. This identification was confirmed by expression cloning in E. coli; we observed HA capsule formation in vivo and detected activity in membrane preparations in vitro. The polypeptide size was verified by photoaffinity labeling of the native P. multocida HA synthase with azido-UDP sugar analogs. Overall, the P. multocida sequence is not very similar to the other known HA synthases from streptococci, PBCV-1 **virus**, or vertebrates. Instead, a portion of the central region of the new enzyme is more homologous to the amino termini of other bacterial glycosyltransferases that produce different capsular polysaccharides or lipopolysaccharides. In summary, we have discovered a unique HA synthase that differs in sequence and predicted topology from the other known enzymes.

L9 ANSWER 12 OF 21 MEDLINE on STN DUPLICATE 2

AU McMahon C W; Traxler B; Grigg M E; Pullen A M
TI **Transposon**-mediated random **insertions** and **site -directed mutagenesis** prevent the trafficking of a mouse mammary tumor **virus** superantigen.
SO Virology, (1998 Apr 10) 243 (2) 354-65.
Journal code: 0110674. ISSN: 0042-6822.
AB Mouse mammary tumor viruses (MMTVs) encode superantigens (Sags) which are critical to the life cycle of infectious virus and can mediate extensive deletion of T lymphocytes when expressed by endogenous proviruses. Little is known about the structure, intracellular trafficking, or nature of Sag association with major histocompatibility (MHC) class II products. In order to gain a better understanding of Sag structure-function relationships, we extensively mutagenized this type II glycoprotein using two different approaches: transposon-mediated random in-frame insertion mutagenesis and site-directed mutagenesis targeting clusters of charged residues. We find that 31 codon insertions are infrequently tolerated in Mtv-7 Sag, with just 1 of 14 insertion mutants functionally presented on the surface of B cells. Surprisingly, similar effects were observed with Sag mutants with substitutions at pairs of charged residues; only 2 of 6 mutants trafficked to the plasma membrane and stimulated T cells, 1 with a temperature-sensitive phenotype. The data suggest that the nonfunctional Mtv-7 Sag mutants are stringently retained in the endoplasmic reticulum due to conformational defects rather than disrupted interactions with MHC class II, thus identifying charged amino acids critical to the structural stability of viral superantigens.

L9 ANSWER 13 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Ehrmann, M.; Bolek, P.; Mondigler, M.; Boyd, D.; Lange, R.
TI TnTIN and TnTAP: Mini-transposons for site-specific proteolysis in vivo
SO PROC. NATL. ACAD. SCI. USA, (19971100) vol. 94, no. 24, pp. 13111-13115.
ISSN: 0027-8424.
AB Tobacco etch **virus** (TEV) protease recognizes a 7-aa consensus sequence, Glu-Xaa-Xaa-Tyr-Xaa-Gln-Ser, where Xaa can be almost any amino acyl residue. Cleavage occurs between the conserved Gln and Ser residues. Because of its distinct specificity, TEV protease can be expressed in the cytoplasm without interfering with viability. Polypeptides that are not natural substrates of TEV protease are proteolyzed if they carry the appropriate cleavage site. Thus, this protease can be used to study target proteins in their natural environment in vivo, as well as in vitro. We describe two Tn5-based mini-**transposons** that insert TEV protease cleavage sites at random into target proteins. TnTIN introduces TEV cleavage sites into cytoplasmic proteins. TnTAP facilitates the same operation for proteins localized to the bacterial cell envelope. By using two different target proteins, Seca and TolC, we show that such modified proteins can be cleaved in vivo and in vitro by TEV protease. Possible applications of the site-specific proteolysis approach are topological studies of soluble as well as of inner and outer membrane proteins, protein inactivation, **insertion mutagenesis** experiments, and protein tagging.

L9 ANSWER 14 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Jenkins, T.M.; Esposito, D.; Engelman, A.; Craigie, R.*
TI Critical contacts between HIV-1 integrase and viral DNA identified by structure-based analysis and photo-crosslinking
SO EMBO J., (19971117) vol. 16, no. 22, pp. 6849-6859.
ISSN: 0261-4189.
AB Analysis of the crystal structure of HIV-1 integrase reveals a cluster of lysine residues near the active site. Using **site-directed mutagenesis** and photo-crosslinking we find that Lys156 and Lys159 are critical for the functional interaction of integrase with viral DNA. Mutation of Lys156 or Lys159 to glutamate led to a loss of both 3' processing and strand transfer activities in vitro while maintaining the ability to interact with nonspecific DNA and support

disintegration. However, mutation of both residues to glutamate produced a synergistic effect eliminating nearly all nonspecific DNA interaction and disintegration activity. In addition, **virus** containing either of these changes was replication-defective at the step of integration. Photo-crosslinking, using 5-iododeoxyuracil-substituted oligonucleotides, suggests that Lys159 interacts at the N7 position of the conserved deoxyadenosine adjacent to the scissile phosphodiester bond of viral DNA. Sequence conservation throughout retroviral integrases and certain bacterial **transposases** (e.g. Tn10/IS10) supports the premise that within those families of polynucleotidyl transferases, these residues are strategic for DNA interaction.

L9 ANSWER 15 OF 21 LIFESCI 
AU Bravo-Angel, A.M.; Becker, H.-A.; Kunze, R.; Hohn, B.; Shen, W.-H.*
TI The binding motifs for Ac transposase are absolutely required for excision of Ds1 in maize
SO MOL. GEN. GENET., (1995) vol. 248, no. 5, pp. 527-534.
ISSN: 0026-8925.
AB A reverse genetic system for studying excision of the transposable element Ds1 in maize plants has been established previously. In this system, the Ds1 element, as part of the genome of maize streak **virus** (MSV), is introduced into maize plants via agroinfection. In the presence of the Ac element, excision of Ds1 from the MSV genome results in the appearance of viral symptoms on the maize plants. Here, we used this system to study DNA sequences required in cis for excision of Ds1. The Ds1 element contains the Ac **transposase** binding motif AAACGG in only one of its subterminal regions (defined here as the 5' subterminal region). We showed that mutation of these motifs abolished completely the excision capacity of Ds1. This is the first direct demonstration that the **transposase** binding motifs are essential for excision. **Mutagenesis** with oligonucleotide **insertions** in the other (3') subterminal region resulted in elements with either a reduced or an increased excision efficiency, indicating that this subterminal region also has an important function.

√

L9 ANSWER 16 OF 21 CAPLUS 
IN Phadnis, Suhas H.; Huang, Henry V.; Berg, Doulgas E.
TI DNA transposon Tn5supf for site-directed mutagenesis and sequencing of cloned DNA
SO U.S., 10 pp. Cont.-in-part of U.S. 5,137,829.
CODEN: USXXAM

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | US 5316946 | A | 19940531 | US 1990-468450 | 19900122 |
| | US 5137829 | A | 19920811 | US 1987-105422 | 19871005 |

AB A small, novel transposon useful for mutagenesis and sequencing DNAs cloned in phage λ is has at each terminus a segment of 19 nucleotides selected from the O-end and I-end sequences of Tn5, at least one restriction enzyme site positioned less than 20 nucleotides from each terminal segment, and a supF amber-suppressor tRNA gene insert. The transposon transposes efficiently and fairly randomly, is easily selectable in the right background, has unique sites to start sequencing from, and is small.

L9 ANSWER 17 OF 21 LIFESCI 
AU Grevelding, C.; Becker, D.; Kunze, R.; Von Menges, A.; Fantes, V.; Schell, J.; Masterson, R.
TI High rates of Ac/Ds germinal transposition in Arabidopsis suitable for gene isolation by insertional mutagenesis.
SO PROC. NATL. ACAD. SCI. USA., (1992) vol. 89, no. 13, pp. 6085-6089.
AB Overexpression of the Activator (Ac) **transposase** gene in Arabidopsis thaliana resulted in a minimal germinal transposition frequency of 27% in which independent Dissociation (Ds) transposition

events were observed. Molecular analysis of 45 F sub(1) generation Ac/Ds plants indicated that high rates of somatic excision had occurred, and independent germinal insertions were identified in F sub(2) generation progeny plans. A tandem cauliflower mosaic **virus** (CaMV) promoter fused to two different Ac coding sequences significantly increased the rate of Ds transposition. The CaMV-Ac fusions activated single and multiple copies of two different Ds elements, DsDHFR and Ds35S-1, and reciprocal crosses resulted in similar transposition frequencies. The improved rate of independent germinal transposition observed makes Arabidopsis) an ideal system for **insertional mutagenesis**
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L9 ANSWER 18 OF 21 LIFESCI COPYRIGHT 2005 CSA on STN
AU Levy, L.S.; Lobelle-Rich, P.A.
TI Insertional mutagenesis of flvi-2 in tumors induced by infection with LC-FeLV, a myc-containing strain of feline leukemia virus.
SO J. VIROL., (1992) vol. 66, no. 5, pp. 2885-2892.
AB LC-FeLV is a myc-containing strain of feline leukemia **virus** (FeLV) which exhibits only partial transforming activity in vitro and in vivo. LC-FeLV infection in kittens may induce, but does not necessarily induce, thymic lymphosarcoma in viremic animals after a short latency. These observations suggest that infection with LC-FeLV is not sufficient to induce complete transformation and that another genetic event(s) is required. One possibility for such an event is that the integrating provirus acts as an insertional mutagen and thereby disrupts the structure or function of another proto-oncogene. Using a strategy of **transposon** tagging, this possibility was examined in eight feline T-cell lymphosarcomas, including four induced by experimental infection with LC-FeLV, three induced by natural infection with FeLV, and one FeLV-negative tumor. The data suggest that interruption of the flvi-2 locus cooperates with the myc oncogene in the induction of T-cell lymphomas by LC-FeLV; indeed, the observations indicate that the **insertional mutagenesis** of flvi-2 plays a role in T-cell lymphomagenesis even in the absence of feline v-myc.

L9 ANSWER 19 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3
AU Weber, Peter C.; Levine, Myron; Glorioso, Joseph C.
TI Rapid identification of nonessential genes of herpes simplex virus type 1 by Tn5 mutagenesis
SO Science (Washington, DC, United States) (1987), 236(4801), 576-9
CODEN: SCIEAS; ISSN: 0036-8075
AB The large genome of herpes simplex virus type 1 (HSV-1) encodes at least 80 polypeptides, the majority of which have no recognized function. A subgroup of these gene products appears to be nonessential for virus replication in cell culture, but contributes to the complex life cycle of the virus in the host. To identify such functions, a simple insertional mutagenesis method has been used for selective inactivation of individual HSV-1 genes. The bacterial transposon Tn5 was allowed to insert randomly into cloned restriction fragments representing the entire short unique (Us) region of the HSV-1 genome. Of the 12 open reading frames that were mutagenized with Tn5, mutant derivs. of US2, US4, and US5 were recombined into the virus. These three genes proved to be nonessential for HSV-1 replication in Vero (African Green monkey kidney) cells and the US4 gene appeared to be involved in viral pathogenesis in the central nervous system of mice. This rapid mutagenesis procedure should prove useful in exploring the entire HSV-1 genome as well as the genomes of other complex animal viruses.

L9 ANSWER 20 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
AU Karlovsky, P.; Vaskova, M.
TI Tn1000 insertional mutagenesis of cloned repressor gene of the phage L: plasmid oligomerization in the presence of F'lac
SO Folia Microbiologica (Prague, Czech Republic) (1987), 32(3), 185-93, 3

plates
CODEN: FOMIAZ; ISSN: 0015-5632

AB An ampicillin-resistance (Apr) plasmid carrying the cloned repressor gene cII on the L phage (Salmonella typhimurium) was transferred by F'lac into an F- recipient. Two types of plasmids were isolated from Apr transconjugants. The majority of plasmids were dimers with 1 copy of Tn1000 inserted, the minority being monomers with 1 copy of Tn1000. This proportion remained unaltered when the F'lac strain transformed with a monomeric form of the recombinant plasmid was used as a donor. An extensive oligomerization of plasmids originating from pBR322 was observed in the presence of F'lac; its relation to transposition-related processes is discussed.

L9 ANSWER 21 OF 21 LIFESCI 

AU Zahm, P.; Hohmeyer, C.; Geider, K.

TI Site-specific mutagenesis of the Ti plasmid by transformation of Agrobacterium tumefaciens with mutagenized T-DNA fragments cloned in E. coli plasmids.

SO MOL. GEN. GENET., (1984) vol. 194, no. 1-2, pp. 188-194.

AB A DNA fragment covering the complete T-region of the Ti plasmid from Agrobacterium tumefaciens strain C58 was cloned in the Escherichia coli cosmid pHC79. This fragment was mutagenized by insertion of **transposon** Tn 5. The isolated DNA from hybrid plasmids was used to transform cells of A. tumefaciens strain C58 applying the freeze-thaw method. Although the E. coli plasmids with the mutagenized Ti plasmid fragment cannot replicate in these cells, they can be rescued by recombination with the homologous region of the Ti plasmid. The cointegrates formed were resolved in a second recombination event, which was detected by loss of the drug resistance marker of the E. coli plasmid. Subcloning of the Ti plasmid fragments labeled with Tn5 showed that the frequency of rescue of the hybrid plasmid as a cointegrate and its segregation in agrobacteria depend on the degree of homology with the Ti plasmid. The authors also applied the strategy for **site-directed** Tn5 **mutagenesis** to **insert** specifically the replication origin of bacteriophage fd and the thymidine kinase gene from Herpes **virus** into the T-DNA of Ti plasmid-C58.

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☐ 2. 20050009060. 07 May 04. 13 Jan 05. Multiplexed multitarget screening method. Beernink, Andrew, et al. 435/6; 435/40.5 C12Q001/68 G01N001/30 G01N033/48.

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☐ 3. 20040253727. 15 Jan 03. 16 Dec 04. COMPOSITIONS AND METHODS FOR MAKING MUTATIONS IN CELL LINES AND ANIMALS. Harrington , John Joseph, et al. 435/455; 435/6 C12Q001/68 C12N015/85.

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☐ 5. 20040253590. 15 Jan 03. 16 Dec 04. COMPOSITIONS AND METHODS FOR MAKING MUTATIONS IN CELL LINES AND ANIMALS. Harrington , John Joseph, et al. 435/6; 435/455 C12Q001/68 C12N015/85.

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☐ 7. 20040203158. 15 Jan 04. 14 Oct 04. Transposon-insulator element delivery systems. Hackett, Perry B., et al. 435/473; C12N015/74.

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